Artificial Incubation of Great Bustard(*Otis tarda*) Eggs

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Abstract　A trial of artificial incubation to great bustard (*Otis tarda*) egg was conducted from 1995 to 1997. Among the 45 collected eggs, 28 eggs were fertilized and 26 hatched, with fertilizing rate 62.2% and incubation rate 92.85%. The mean Incubation-days for great bustard egg was confirmed as 24.4(21-28 days) with the formula for caculating fresh egg weight ($W=KwLB^2$) and that for counting the incubated days by parents birds in field ($Id=24(w-y)/0.144y$). The proper incubation temperature and relative humidity were 36-37.8 °C and 50-65%, respectively. The egg weights and egg size average 130.45 g and 77.4±1.42 × 55.5 ± 0.65 mm respectively. The total weight loss of egg was 18.38±0.646 g, daily weight loss 0.748±0.071g in the incubation time, with a weight loss rate of 13.6±1.02%. A linear regression equation to discribe the relationship between the egg weight and incubation days, was built, $y=130.73-0.619x$ (x—incubation day, y—egg weight), r=-0.978. Twenty-eight hours were nassassary for great bustard embryo to complete the fledging when the were put in gas room. The mean weight of fledglings was 86.3±3.29 g(n=26).

Key words: *Otis tarda*, Great bustard, Egg, Artificial incubation

Introduction

Great bustard(*Otis tarda*), one of the endangered birds in the world, was defined as first rank protected animal in China It is estimated that about 2000-3000 individuals are surviving in China. Artificial incubation is one of the important way to restore and save such endangered species as great bustard. But, no reports were found on incubation of great bustard in domestic. During 1995-1997, we conducted the experiment on artificial incubation of great bustard egg.

Material and Method

Source of eggs

The experimental eggs for artificial incubation were collected in Xing'an League grassland, Inner Mongolia. They were put into a incubation can with thermobag, fixed with absorbent cotton and foamed plastics, Under the controlled temperature of 35-38 °C, they were sent to incubation place within 12 h.

Incubation requirements

The collected eggs, 60% of which were already incubated by parents, were labeled by the size and weight before incubating. The formula for calculating fresh egg weight, $W=KwLB^2$ (Hoyt 1979), and that for calculating the incubation days by parents in the field,

$Id=24(w-y)/0.147y$ (Burnham 1983), were adopted to confirm the incubated days and stage of each wild eggs precisely.

9F-552 incubator made by Wuxi Incubator Manufactory was used in our experiment, which can control the temperature and humidity automcitically. The incubation temperature was controlled at 36-38 °C, and the humidity at 50-65%. The eggs were turned over with 90° along the longitudinal axle every two hours, simutaneously, cooled for 2-5 min. In the later incubating period (after 17 days), the eggs were cooled outside the incubation box twice every day, for 10 min each time. The eggs were weighed every two days and were tested with warm water or plastic plate every five days. It was difficult to watch the embryo conditions with light and egg illuminator because the eggshell of great bustard egg is relative thick. So the embryo's growing and moving condition were examined according to the temperature change of egg surface, accompanied with those two physical methods discussed above. The incubation period was divided into four phases, early stage, middle stage, later stage and fledging period. It is suggested that the incubator should be sterilized with $KMnO_4$, However, with consideration of incubation rate being affacted, the eggs collected from the field were not sterilized in our experiment.

Results

From 1995 to 1997, total 45 great bustard egg were artificially incubated. Their size, weight, character were described in Table I.

The shape of great bustard egg is like oval. In color, it shows dark greyish green, light greyish green, light brown, bark brown, and accompanied with dark brown irregular stripes and spots. The weights of the largest and smallest eggs was 161.6 g and 88.3 g, respectively. The egg diameter for the maximum was 84.8×59.0 mm, and for the minmum 69.0 × 48.7 mm The mean weight of these eggs was 130.5±7.11g and the mean egg diameter was 77.4±1.42 × 55.8±0.654 mm. It was verified that the egg size was relative to ages and individual difference of the parents, while color is related to habitat .

Table 1. Measurement of Great bustard egg

Trails		Weight (g)	Length (mm)	Breadth (mm)	Egg shape index*
1995	average±SD	139.5±5.096	78.4±0.19	57.0±0.97	1.376±0.0200
n=2	range	135.2-143.1	78.2-78.5	56.2-57.7	1.360-1.3914
1996	average±SD	138.7±8.82	79.8±2.09	56.3±1.20	1.418±0.025
n=8	range	122.2-151.6	76.3-83.5	54.1-57.6	1.373-1.452
1997-1	average±SD	130.7±16.51	76.8±4.37	55.6±2.23	1.381±0.47
n=22	range	88.3-161.6	68-85.2	48.7-59.0	1.277-1.449
1997-2	average±SD	123.6±8.20	76.7±3.22	54.1±1.09	1.417±0.527
n=13	range	102.3-135.8	70.2-81.2	53.0-56.5	1.298-1.526
$\overline{X}$		130.45	77.38	55.35	1.398

* Egg shape index is the length divided by breadth

Incubation temperature and humidity

In the incubation experiment of 1995-1996, the temperature was controlled at 36.5-37.8°C, the humidity at 55-65%; In 1997, the temperature and humidity for the first group of eggs were at 36-37.5°C, 50-65% respectively, and these tow foctors for second group were 36-38°C and 55-65%(see Table 2).

From first day to eleventh day, It was defined as early stage of incubation, in which the temperatures of egg surface and surroundings were the same. From 12th to 17th day were defined as middle stage. In this stage, the embryo was formed and began to move, and the temperature of egg surface was 0.2-0.5°C higher than that of the surroundings. From 17th day to the time the embryo is sent to the gas room, it was defined as later stage. In the later stage, the embryo metabolism was exuberant, and since the temperature of the egg surface was 2-3.1°C higher than that of the surroundings, the eggs need to be moved out of the incubator every day for 20 min cooling to prevent the embryo from thermal death. Fledging period starts from the embryo entering gas room to completely hatchted. In this period the temperature of egg surface was 1.5-2.0°C higher than that of the surroundings.

The artificial incubation experiment of great bustard eggs was conducted for 3 years and 26 fledglings were obtained. It is proved that the suitable incubation temperature to great bustard is 36-37.8°C, the humidity is 50-65%.

Table 2. The Experimental Records of artificial Incubation to great bustard eggs(1995-1997)*

Trails	Egg num.	Tmperature (°c)								RH (%)	Cooling times	Fertilie eggs	Fertilization rate (%)	Fledging number	incubation rate (%)
		early stage		middle stage		later stage		fledging stage							
		surround ings.	egg surface	surround-ing	egg surface	surround-ings	egg surface	suround ing	egg surface						
95.5.30	2	37.8	37.8	37.8	37.8	37	38	36.5	37	55-65	12	1	30	--	0
96.6.3	8	37.8	37.8	37.8	38.9	37	39	36.5	37-37.5	55-65	12	8	100	8	100
97.6.11	22	37.5	37.5	37.0-37.5	37.5-37.8	36-36.5	38-39.5	36.0	37-38.0	50-65	12	11	50	10	90.9
97.6.18	13	37.8-38	37.8	37.8-38	37.8-38	36.5-37	38.5-39	36.5	37.0	55-65	12	8	61.5	8	100

Total incubated eggs	Total fertilized eggs	Rate of fertilization(%)	Total fledging number	Rate of incubation(%)
45	28	62.2	26	92.85

* The eggs were collected in Xing'an League Grassland Inner Mongolia; -- died in later period

Determination of fertilized eggs

The Great bustard eggshell is 600-700 μm in thickness and is untransparent. It was impossible to watch the embryo's developing situation inside the egg by illuminating apparatus. 11-17 days after the eggs were incubated, they were put into a container filled with warm water about 36-37°C. If the eggs fell down to the bottom of the vessel and shook slightly, it means that they were fertilized; if not, they were unfertilized. 18 days later, according to the egg temperature, the eggs were put into the correspondingly warmed glass plate or warm water to determine weather the eggs grew well or not. Under ordinary conditions, It means that the embryo develops well if the eggs sway in water obviously. If the eggs sway slowly or stand still, it indicates that the embryo might be dead in the later stage. But during 24 h period before the embryo enter the gas room, the embryo move weakly or stand still.

Incubation period

It was reported abroad that the incubation period of great bustard egg is 21-28 days. In China, it was 30-31 days (Yu Changyun 1983), 31-35 days (Li Lin 1989), 24-27 day (Li Jinlu 1994), and 22-26 days(Sun sen 1995).

Tracking trials were conducted with 26 fertilized eggs. These trials were divided into 3 groups: artificial incubation, artificial incubation combined with mother birds incubation, and natural incubation. The result showed that the incubation period was 21-28 days, the mean time was 24.45 days, the longest and shortest incubation time was 28 days and 21 days respectively (see Table 2).

Table 3. the Artificial Incubation condition of Great bustard eggs(1996-1997)

Egg No.	Incubating time	caculated weight (g)	incubating weight (g)	egg lenght (mm)	egg breadth (mm)	weight loss before incubation (w-y) (g)	days of mother bird incubation	days of artificial incubation	total days of incubation
1996-1	Jun. 3,1996	137.9	120.0	79.8	56.2	17.9	21	2	23
1996-2		126.7	109.0	77.9	54.5	17.7	23	2	25
1996-6		122.2	106.0	76.3	54.1	16.2	22	3	25
1996-4		142.1	142.0	78.8	57.4	0.1	0	21	21
1996-5		142.1	141.0	79.6	57.4	1.1	1	24	25
1996-6		151.6	150.0	83.5	57.6	1.64	2	21	23
1996-7		143.0	125.0	80.4	57.0	18.0	20	6	26
1996-8		144.2	128.0	82.2	56.6	16.2	18	7	25
1997-1-4	June 11, 1997	124.9	124.0	73.3	55.8	0.93	1	24	25
1997-1-6	(14:00)	154.4	150.0	84.2	58.1	4.4	6	15	21
1997-1-7		137.8	132.0	74.3	58.2	5.8	7	19	26
1997-1-8		130.3	128.0	73.5	56.9	2.3	3	20	23
1997-1-10		127.4	122.0	77.8	54.7	5.4	7	17	24
1997-1-12		122.5	114.0	74.8	54.7	8.5	11	17	28
1997-1-13		114.1	114.0	71.5	54.0	0.1	0	24	24
1997-1-14		128.8	120.0	74.5	56.2	8.8	11	15	26
1997-1-15		118.5	111.0	74.8	53.8	7.5	10	16	26
1997-1-16		136.3	128.0	78.3	56.4	8.3	10	15	25
1997-2-2		114.7	112.0	74.0	53.2	2.7	4	17	21
1997-2-3		125.2	114.0	79.0	53.8	11.2	15	11	26
1997-2-4		131.5	120.0	81.2	54.4	11.5	14	11	25
1997-2-5		122.8	111.0	72.3	55.7	11.8	17	8	25
1997-2-7		135.8	124.0	77.7	56.5	11.8	14	7	21
1997-2-9		135.4	120.0	80.0	55.6	15.4	18	7	25
1997-2-12		124.4	116.0	80.9	53.0	8.4	11	14	25
1997-2-13		102.3	92.0	70.2	51.6	10.3	16	8	24
$\bar{X}$									24.35

$W=Kw \cdot L \cdot B^2$ $Kw=0.005474$. $Id =w-y/0.147/24$

Weight loss and weight loss rate

From Table 4, we could conclude that the weight of great bustard eggs had changes during incubation period. That is, the eggs weight reduced regularly from the beginning of incubation to fledging. Egg weight relates to incubation days. A linear regression equation was set up for expressing the relation between egg weight and incubation days, $y=130.73$-

0.619x, r = -0.978 (x is incubation days; y is egg weight).

The total weight loss of egg was 18.37± 0.646g, the weight loss rate 13.6±1.02%, the daily weight loss 0.748±0.071 g, and the daily weight loss rate was 0.578±0.074%.

Fledging weight and their remains

Some remains including the eggshell, unabsorbed albumen of eggs and fetus feces, were left inside the eggs when fledgling came out of egg. In 1996-1997, 26 successful fledglings were obtained. The remains are recorded in Table 5.

Table 4. The weight Loss of great bustard eggs(1996-1997)

Trail year		Weight of fresh eggs(g)	Weight before fledging (g)	Total weight loss (g)	Rate of weight loss	Weight loss of one day	rate of weight loss one day
1996	X(SD)	127	123.25	18.18	14.3%	0.78	0.58
	R106-150	109-140	11.6-31.2	7.7%-21.6%	0.5-1.25	0.335-0.76	
1997	X(SD)	124.04	109.1	19.4	14.5	0.846	0.65
	R111.0-150.0	94.0-123.0	14.3-31.4	10.5-24.6	0.518-1.59	0.42-1.025	
1997	X(SD)	124.8	114.7	17.55	12.1	0.62	0.506
	R102.3-135.8	90.0-123.0	12.3-20.4	9.1-16.4	0.49-0.816	0.364-0.58	
Total	X(SD)	125.28±0.888	115.68±4.16	18.37± 0.646	13.6±1.02	0.748± 0.071	0.578±0.074
	R106.43-145.26	97.67-128.67	12.73-27.67	9.1-20.9	0.503-1.213	0.373-0.788	

1996: y=138.8-0.638x, r=-0.985; 97-2: y=124.0-0.388x, r = -0.971; 1997-1: y=129.5-0.421x, r=-0.975,
Final equation: y=130.73-0.619x, r = -0.978

Table 5. Records in weights of 26 great bustard eggs

Egg No.	Weight of chicks (g)	Remains weight of egg (g)	Weight of fresh egg (g)	Weight of eggs loss (g)	Ratio of chick weight and fresh eggs
1996-1	92	20	137.9	13.9	66.7
1996-2	82	20	126.7	17.7	64.7
1996-3	82	18	122.2	15.2	67.1
1996-4	100	18	142.1	17.1	70.4
1996-5	94	18	142.1	16.3	66.2
1996-6	107	18	151.6	18.6	70.1
1996-7	96	16	143.0	23	67.1
1996-8	96	16	144.2	25.2	67.0
1997-1-4	83	18	124.93	14.9	66.0
1997-1-6	90	27	154.4	28.4	58.3
1997-1-7	85	25	137.7	23.7	62.0
1997-1-8	82	18	130.3	16.3	62.9
1997-1-10	74	22	127.4	25.4	58.1
1997-1-12	80	18	122.5	14.5	65.3
1997-1-13	74	17	114.13	20.1	64.8
1997-1-14	84	24	128.8	18.8	65.0
1997-1-15	78	23	118.5	18.5	65.8
1997-1-16	94	18	136.3	18.3	68.9
1997-2-2	80	17	114.7	12.7	69.7
1997-2-3	82	22	125.2	15.2	65.5
1997-2-4	88	19	131.5	15.5	67.0
1997-2-5	90	24	122.8	17.8	73.0
1997-2-7	92	22	135.8	15.8	70.0
1997-2-9	84	22	135.4	15.4	69.4
1997-2-12	87	19	124.4	20.4	70.0
1997-2-13	68	20	10.23	12.0	66.0
$\bar{X}$ (n=26)	86.8±0.346	19.96±0.56	130.7±2.31	18.1±0.81	66.4±3.9

Among 26 fledglings, the largest fledgling weighed 107 g, taking up 70.1% of the fresh egg weight. The smallest one weighed 68 g, taking up 66% of the fresh egg weight. The average weight of 26 fledglings was 86.8±0.346 g, taking up 66.4±0.039% of the weight of fresh eggs. The mean remains weight of these eggs was 19.69±0.56g.

Fledging behavior

Successful fledging depends on incubation condition and the quantity of eggs. Among 26 eggs, only two eggs presented scratching behavior of embryo in later incubation period before they entered the gas room. Other embryos entered the gas room directly. It was found in the test that the embryo stopped moving or moved slightly in 12 to 24 h just before its entering the gas room. When they entered the gas room, the low voice of "zha-zha" could be heard. At this time, we figured out the location on gas room, stopped turning over the eggs and moved them to fledging room. With the time went on, the voice of fledging and the rhythm of pecking eggshell became stronger and stronger. This phenomena indicated the embryo growing well. If the voice or the rhythm is weak, it mean that the embryo is weak.

The normal pecking place of chicks is at the obtuse end of the egg. In this experiment, the pecking site of 28 eggs was 25-30 mm away from the obtuse end of

the eggs, except for only 1 egg is at the middle area because of the embryo's abnormal position. The shape looks like crack with the tiny hole of 40×60 mm.

Normaly, fledglings peck the obtuse end of egg. About 30-50 min before the fledgling is born, they try their best to peck the eggshell along the anti-clock wise direction continuously and regularly. At last, the fledgling struggles 3-5 times to move out of the egg when the eggshell about 40-50 mm around the egg is left unbroken. At that time, the sound of "ka-ka" coming from the broken eggs could be heard. The fledgling's head and left wing coming out of the egg first, then the right wing, neck, breast, feet, abdomen and the tarsus. From the chick entering the gas room to peck shell, the longest time was 18 h, the shortest time 2 h, the mean time 11.2 h. The longest time from pecking shell to fledging is 29 h and 45 min, the shortest time 6 h, and the mean time is 16 h and 48 min(see Table 6).

Table 6. 1996-1997 fledging condition of Great bustard chicks

Egg No.	Entering going gas room or crying time		Time of pecking shell		Time of interval from crying to pecking shel (h)l	Fledging time		time of interval from pecking to fledging
1996-1			Jun. 3	4:30		Jun.4	4:00	23.5
1996-2			Jun. 3	10:45		JUn.4	4:00	17.25
1996-3			Jun.4	6:00		Jun.4	16:30	10.5
1996-4	Jun. 21	14:00	Jun. 22	4:00	14	Jun. 22	16:00	12
1996-5	Jun. 23	14:00	Jun. 24	6:00	16	Jun. 25	4:00	22
1996-6			Jun. 21	14:30		Jun. 22	14:00	24
1996-7			Jun. 8	8:30		Jun. 8	20:25	12.5
1996-8			Jun. 8	10:00		Jun. 9	15:45	29.45
1997-1-4	Jul. 02	12:00	Jul. 3	2:00	14	Jul. 3`	20:00	18
1997-1-6	Jun. 23	10:00	Jun. 24	0:00	14	Jun.. 24	8:30	8.5
1997-1-7	Jun. 27	10:00	Jun. 28	4:00	18	Jun. 28	10:00	6
1997-1-8	Jun. 28	8:00	Jun. 28	12:00	4	Jun. 29	8:00	20
1997-1-10	Jun. 25	10:00	Jun. 25	14:00	4	Jun. 26	6:00	16
1997-1-12	Jun. 25	8:00	Jun. 25	14:00	2	Jun. 26	2:00	16
1997-1-13	Jul. 02	16:00	Jul. 3	2:00	10	Jul. 3	22:00	20
1997-1-14			Jun. 24	6:00		Jun. 24	21:00	15
1997-1-15	Jun. 24	8:00	Jun. 24	14:00	6	Jun.25	6:00	16
1997-1-16	Jun.23	10:00	Jun. 24	3:00	16	Jun.24	20:15	18.25
1997-2-2	JuL.03	4:00	Jul. 3	16:00	12	Jul.4	10:00	18
1997-2-3	Jun.27	10:00	Jun. 27	20:00	10	Jun.28	13:30	17.5
1997-2-4	Jun.28	14:00	Jun. 29	2:00	12	Jun.29	18:00	16
1997-2-5	Jun.24	8:00	Jun. 24	22:00	14	Jun.25	17.45	19.75
1997-2-7	Jun.23	18:00	Jun. 24	2:00	8	Jun.24	18:00	16
1997-2-8	Jun.23	10:00	Jun. 24	2:00	16	Jun.24	14:00	12
1997-2-12	Jun.30	8:00	Jun. 30	16:00	8	Jul.1	10:00	18
1997-2-18	Jun.24	8:00	Jun. 24	22:00	14	Jun.25	12:00	14
$\bar{X}$					11.2			16.8

Summary and Discussion

The mean weight of Great bustard egg is 137±7.11 g, the mean egg diameter is 77.9±0.74×55.8±0.65 mm,

which is heavier than the record reported by Yu Changyun (1983), and lighter than the record reported by Li Lin (1989).

Both the formula of calculating the fresh egg weight,

which was put forward by Hoyt (1979), and that of calculating the incubated days by parents birds, which was put forward by Bunham (1983) are fit for great bustard egg.

Egg shape index of great bustard egg is 1.39, which is similar to most of the birds Elongation = 1.300 (Deeming 1979).

The mean artificial incubation period of great bustard egg is 24 days.

The artificial incubation period can be divided into four stages: early stage (1-11 days), middle stage (12-16 days), later stage (19-22 days) and fledging period (23-28 days).

From 1995 to 1997, 45 eggs were incubated and 26 fledglings were obtained, The incubation rate was 92.85%. We suggest that the incubation temperature is 36-37.8°C, and the relative humidity is 50-65%.

From 1996 to 1997, 28 fertilized eggs were artificially incubated and 26 fledglings were gained. The mean weight loss is 13.6±1.02%, the average daily weight loss is 0.578±0.074%.

11 h and 12 min, an average time, is required from fledging entering the gas room to pecking the eggshell, 16 hours and 48 min from pecking eggshell to fledging. The total time of fledging is about 28 hours. The average body eight of great bustard fledglings is 86.8 ±0.346g(n=26).

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